Analysis of Clozapine, Nortriptyline, Paroxetine and Zolpidem Using Dried Blood Spots

Application Note

Pharmaceuticals

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Introduction

Dried blood spotting (DBS) has been gaining in popularity in recent years for pre-clinical and clinical trials for new chemical entities in the pharmaceutical industry. Pharmaceutical companies have been using it to sample, store, and test blood samples for analytes. 10 µL of blood is spotted onto paper and allowed to dry for at least 2 hours. A 2–4 mm core is punched out of the spot. The analytes are desorbed from the paper using solvent and analyzed by LC-MS/MS. Typically, cellulose materials have been used for these applications, but a new non-cellulose material is now available. This dried matrix spotting (DMS) card is effective for storing and preparing compounds in biofluids. The data demonstrate good analytical results for blood, but any dried biological fluid can be analyzed by this technique.

Experimental

Four analytes were chosen based on the availability of deuterated standard: Clozapine, Zolpidem, Paroxetine and Nortriptyline.

Precision and accuracy recoveries were calculated based on the linear regression of the calibration standards. In order to verify the accuracy and precision of the calibration curve, a mid-level (5.0 ng/mL) and high level (500 ng/mL) were chosen.

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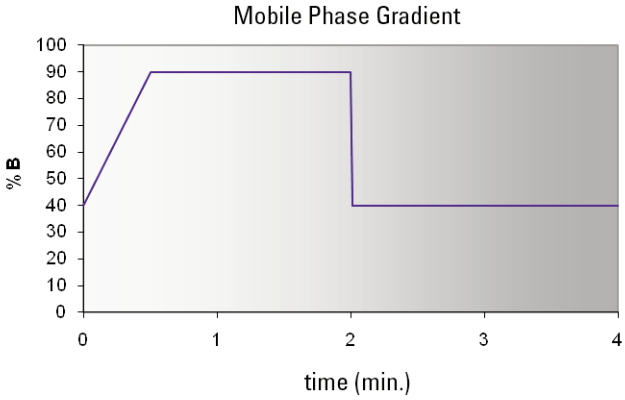
LC-MS conditions

Column	Agilent Pursuit XRs^{ultra} C18, 50 mm × 2.0 mm	
Mobile phase	A: 0.1% Aqueous Formic Acid	
	B: MeOH	
Pump program	Flow rate 200 µL/min.	
	t_0	A: 60%, B: 40%
	$t_{0.5-2.0}$	A: 10%, B: 90%
	$t_{2.01-4:00}$	A: 60%, B: 40%
Run time	4:00 minutes	
Gas temp	350 °C	
Gas flow	10 l/min	
Nebulizing	20 psi	
Pol	Pos	



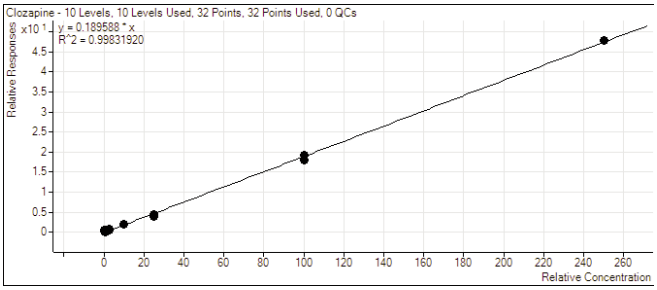
Compound	Q1 ion	Product ion	CE
Zolpidem	308.2	235.1	36 V
Zolpidem-D6	314.2	235.0	36 V
Clozapine	327.1	270.1	20 V
Clozapine-D4	331.2	272.1	20 V
Paroxetine	330.2	192.1	20 V
Paroxetine-D6	336.2	198.1	20 V
Nortriptyline	264.2	105.1	16 V
Nortriptyline-D3	267.2	233.0	8 V

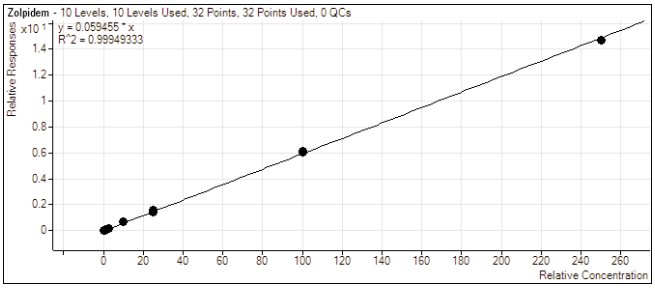
Results and Discussion

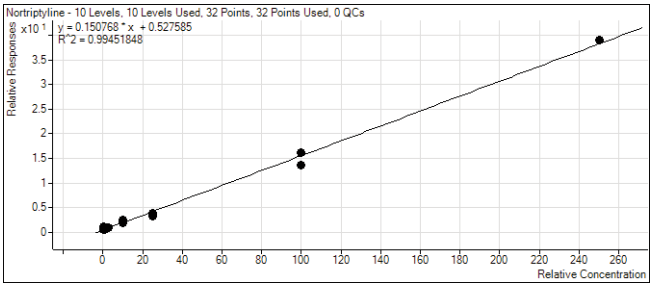
1 mL human blood was spiked with 10 µL of each working standard to create a calibration curve of 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 20, 50, 200, and 500 ng/mL. 15 µL of each of these standards was spotted onto an Agilent Bond Elut DMS card.

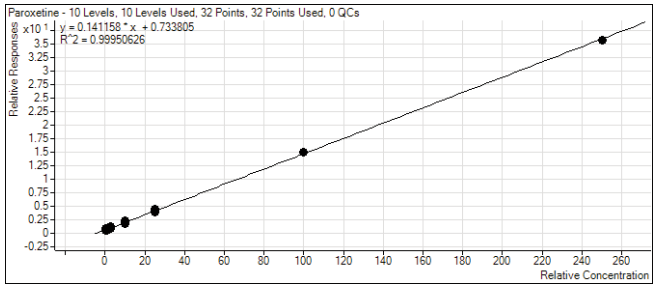
A 3 mm disk was punched and placed into a 96-well plate. 300 µL of 0.1% formic acid in 80% methanol (with 0.066 ng/mL of deuterated internal standard mix) was added to each well and vortexed.

The samples were evaporated to dryness and reconstituted in 100 µL of mobile phase.


Clozapine - 10 Levels, 10 Levels Used, 32 Points, 32 Points Used, 0 QCs
y = 0.189588 * x
R^2 = 0.99831920
Relative Responses
Relative Concentration

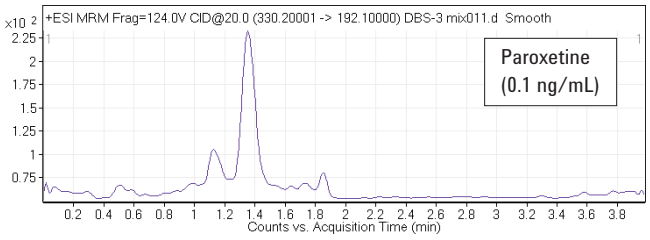

Zolpidem - 10 Levels, 10 Levels Used, 32 Points, 32 Points Used, 0 QCs
y = 0.059455 * x
R^2 = 0.99949333
Relative Responses
Relative Concentration

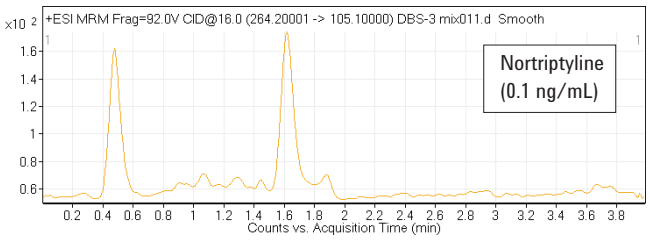

Nortriptyline - 10 Levels, 10 Levels Used, 32 Points, 32 Points Used, 0 QCs
y = 0.150768 * x + 0.527585
R^2 = 0.99451848
Relative Responses
Relative Concentration

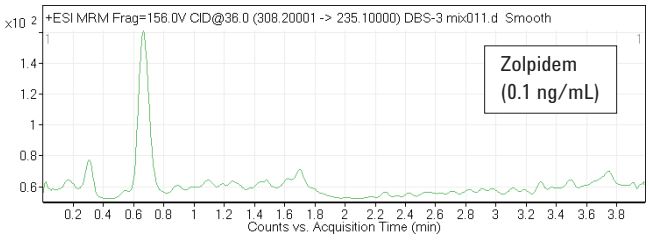

Paroxetine - 10 Levels, 10 Levels Used, 32 Points, 32 Points Used, 0 QCs
y = 0.141158 * x + 0.733805
R^2 = 0.99950626
Relative Responses
Relative Concentration

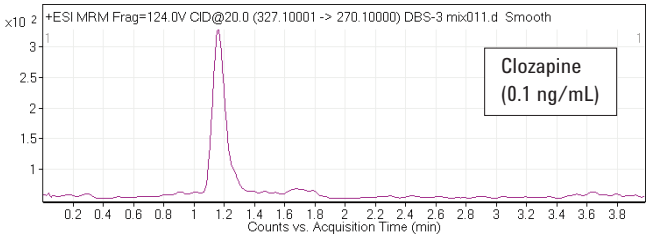
Analyte Recoveries (n=6)

	Concentration	% Rec	RSD
Zolpidem	5.0 ng/mL	102%	6%
	500 ng/mL	110%	6%
Clozapine	5.0 ng/mL	98%	5%
	500 ng/mL	110%	4%
Paroxetine	5.0 ng/mL	102%	6%
	500 ng/mL	110%	6%
Nortriptyline	5.0 ng/mL	89%	7%
	500 ng/mL	92%	7%









Conclusions

Four compounds in blood were successfully desorbed and analyzed by LC-MS/MS. Good detection levels were achieved using an Agilent 1290 LC and 6460 Mass Spectrometer. Linearity was demonstrated using a 1st order regression and correlation coefficients were better than 0.995. Relative recoveries were within 10% of the true value and RSDs less than 10%. The Agilent Bond Elut DMS card proved to be an effective means of storing blood samples as well as crashing out any unwanted proteins for a simple approach to sample preparation prior to
LC-MS/MS.

For More Information

Bond Elut DMS cards are for use in DMPK/ADME research applications only. They should not be used in diagnostic procedures.

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Printed in the USA
June 29, 2011
5990-8033EN

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